bio-protocol Preprint

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Mitochondria isolation

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An abbreviated version of this protocol was published in eLIFE in May 2021

Genome-wide CRISPRi screening identifies OCIAD1 as a prohibitin client and regulatory determinant of mitochondrial Complex III assembly in human cells

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Detailed protocol

Mitochondria isolation protocol for U2OS cells (adherent)

Homogenization buffer (adjusted to pH 7.4 at 4^{o}C):

- 10 mM HEPES
- 1 mM EDTA
- 210 mM mannitol
- 70 mM sucrose

Procedure:

1. Grow U2OS cells to confluency in 150mm Petri dishes.
2. Wash cells 3x with 15ml of ice-cold homogenization buffer.
3. Aspirate residual homogenization buffer from the last wash and scrape cells in 0.75ml of ice-cold homogenization buffer supplemented with 1× protease inhibitor cocktail (MilliporeSigma, Burlington, MA).
4. Transfer cell suspension to a glass Dounce homogenizer fitted with a tight pestle and lyse cells with 6-8 slow strokes. Assess cell lysis efficiency by mixing equal volumes of cell homogenate and 0.4% trypan blue solution and looking under a microscope. About 95% of the cells should be properly lysed without showing sign of nuclei damage.
5. Centrifuge the homogenate at ~1000 g for 5 min at 4^{o}C to pellet nuclei, unbroken cells, and large cellular debris.
6. Carefully collect the supernatant without disturbing the pellet and centrifuge at ~14,000 g for 10 min at 4°C.
7. Carefully discard the supernatant without disturbing the crude mitochondrial pellet and resuspend the pellet in homogenization buffer supplemented with 1× protease inhibitor cocktail. Use ~100ul of homogenization buffer for each 150mm plate of confluent U2OS cells.
8. Aliquot and snap freeze on liquid nitrogen.
9. Store aliquots at -80^{o}C until needed.

Mitochondria isolation protocol for K562 cells (suspension)

Swelling buffer (adjusted to pH 7.4 at 4^{o}C):

- 10 mM HEPES
- 1 mM EDTA

2x homogenization buffer (adjusted to pH 7.4 at 4^{o}C):

- 10 mM HEPES
- 1 mM EDTA
- 420 mM mannitol
- 140 mM sucrose

Homogenization buffer (adjusted to pH 7.4 at 4^{o}C):

- 10 mM HEPES
- 1 mM EDTA
- 210 mM mannitol
- 70 mM sucrose

Procedure:

1. Grow 75ml of K562 cells to confluency (~1 million cells per ml) in 150mm Petri dishes.
2. Centrifuge cells at 1000 g for 5 min at room temperature. Remove supernatant.
3. Resuspend cell pellet in 50ml of ice-cold homogenization buffer.
4. Centrifuge cells at 1000 g for 5 min at 4^{o}C. Remove supernatant.
5. Resuspend cell pellet in 2ml of swelling buffer supplemented with 1× protease inhibitor cocktail (MilliporeSigma, Burlington, MA).
6. Incubate on ice for 20min.
7. Transfer cell suspension to a glass Dounce homogenizer fitted with a tight pestle and lyse cells with 25 slow strokes.
8. Immediately add 2ml of 2x homogenization buffer supplemented with 1× protease inhibitor cocktail (MilliporeSigma, Burlington, MA).
9. Centrifuge the homogenate at ~1000 g for 5 min at 4^{o}C to pellet nuclei, unbroken cells, and large cellular debris.
10. Carefully collect the supernatant without disturbing the pellet and centrifuge at ~14,000 g for 10 min at 4°C.
11. Carefully discard the supernatant without disturbing the crude mitochondrial pellet and resuspend the pellet in homogenization buffer supplemented with 1× protease inhibitor cocktail. Use ~400ul of homogenization buffer for 75ml of confluent K562 cells.
12. Aliquot and snap freeze on liquid nitrogen.
13. Store aliquots at -80^{o}C until needed.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Nunnari, J. (2021). Mitochondria isolation. Bio-protocol Preprint. bio-protocol.org/prep1320.
2. Le Vasseur, M., Friedman, J., Jost, M., Xu, J., Yamada, J., Kampmann, M., Horlbeck, M. A., Salemi, M. R., Phinney, B. S., Weissman, J. S. and Nunnari, J.(2021). Genome-wide CRISPRi screening identifies OCIAD1 as a prohibitin client and regulatory determinant of mitochondrial Complex III assembly in human cells. eLIFE. DOI: 10.7554/eLife.67624